Schwann Cell Remyelination and Recurrent Demyelination in the Central Nervous System of Mice Infected With Attenuated Theiler's Virus

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Theiler's murine encephalomyelitis virus (TMEV) infection produces a chronic demyelinating disease in mice, and myelin breakdown appears to be immune-mediated. By using an attenuated TMEV strain, WW virus, to infect mice, the course of the disease was slowed and the severity of the inflammatory and glial responses was reduced. In this circumstance, most of the demyelinating lesions showed extensive remyelination, predominantly by Schwann cells. In addition, it was demonstrated that there was recurrent demyelinating activity in the central nervous system (CNS) of infected animals. It is suggested that the rapidity and intensity of demyelinating lesions may influence the potential for remyelination and that Schwann cell participation may be a more important mechanism of myelin repair than it is now thought to be. The fact that there is recurrent demyelination in TMEV infection increases its relevance as an experimental animal model for multiple sclerosis. (Am J Pathol 98:101-122, 1980)

CERTAIN STRAINS of Theiler's murine encephalomyelitis virus (TMEV) produce a persistent central nervous system (CNS) infection in the natural host.[1] In studies of the pathogenesis of this infection we showed that the white matter lesions consisted of areas of primary demyelination in association with mononuclear cell infiltrates,[2,3] a pattern similar to experimental allergic encephalomyelitis (EAE), the prototype autoimmune disease of the CNS.[4-8] In later experiments, it was demonstrated that demyelination could be prevented by immunosuppression, suggesting that myelin breakdown is immune-mediated.[9]

The earlier studies of this infection were accomplished using brain-derived stocks of TMEV. In this setting infected mice develop a biphasic pattern of CNS disease: an initial phase of gray matter involvement, producing flaccid paralysis, followed by white matter involvment, leading to spastic paralysis. After the adaption of TMEV to tissue culture, these viruses were found to produce the late neurologic disease without antecedent poliomyelitis.[10] In this situation, spastic paralysis occurred after a lengthy incubation period.[10]

We shall now describe the pathologic changes in the CNS of outbred

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mice using one of the tissue-culture adapted TMEVs, WW virus. While gray matter involvement was not observed, a slowly evolving demyelinating process involved the spinal cord white matter. Extensive remyelination occurred as the result of unusually active participation by Schwann cells, and later there was recurrent demyelination, which was best observed in areas restored with peripheral nervous system (PNS) myelin. These findings are supported by our recent discovery of a similar process in C3H/He mice infected with brain-derived DA virus, in which the white matter changes also evolved quite slowly.[11] It is suggested that CNS remyelination may depend in large part on the rapidity and intensity of the preceding wave of myelin breakdown. In addition, Schwann cells may prove to be important in the repair of CNS myelin in other virus-induced demyelinating diseases. Finally, the fact that there is recurrent demyelination in TMEV infection of mice makes this an even more relevant animal model of multiple sclerosis.

Materials and Methods

Animals and Animal Inoculations

Outbred Swiss male mice (CD-1) were purchased from Charles River Breeding Laboratories (Portage, Mich). Mice were housed 5–10 per polycarbonate cage and given food and water *ad libitum*. Animals 4–6 weeks old were inoculated into the right cerebral hemisphere with 10^3–10^4 plaque-forming units (PFU) of WW virus.

Virus

WW virus was adapted to grow and produce a cytopathic effect in tissue culture as described,[10] and a virus stock was prepared on the fourth passage in primary baby mouse kidney cells.

Morphologic Features

Two to 4 mice were sacrificed on Days 16, 24, 31, 44, 84, and 163. Anesthetized animals were perfused with chilled 3% glutaraldehyde in phosphate buffer (pH 7.3) through the left ventricle. Spinal cords were removed, sectioned at approximately 1-mm intervals, and postfixed in 1% osmic acid for 1 hour. Following dehydration in a graded series of ethyl alcohol, sections were cleared in propylene oxide and embedded in Epon. One-micron–thick sections were trimmed for ultrathin sectioning, and the grids were stained with uranyl acetate and lead citrate and then viewed in a Philips 200 electron microscope.

Results

Clinical Observations

The mice were examined daily for the first month and then weekly thereafter. No evidence of clinical disease was observed until 11 weeks, when the majority of animals began to develop spasticity, indicating white matter disease. At no time were animals observed to develop flaccid paralysis, which would be due to gray matter involvement.

Pathologic Observations

Light Microscopy

No pathologic changes were found in the spinal cord gray matter between Days 16 and 163, whereas widespread, scattered lesions were seen in white matter throughout this study. The lesions on Day 16 started as focal perivascular mononuclear cell infiltrates in the lateral columns, around which small groups of naked axons were identified. These inflammatory lesions appeared to progress slowly, because animals sacrificed on Days 24 and 31 showed lesions of similar severity (Figure 1). However, on Day 31 both lateral and anterior columns, and occasionally the posterior columns, were involved. The largest lesions were adjacent to the anterior root entry zone and extended deeply into the cord. Only a moderate number of macrophages were present at these times.

By Day 44, many demyelinated areas appeared inactive or quiescent, since they contained few inflammatory cells and rarely macrophages. Interestingly, at various depths in most lesions numerous Schwann cells were found in contact with naked axons (Figure 2).

At 84 days, remyelination was striking in the anterior and lateral column lesions. The most extensive remyelinated plaques extended beyond half the thickness of the lateral columns and for a considerable distance from the anterior median sulcus in the anterior columns. Remyelinated areas had a lobular appearance due to development of numerous fibrous septums (Figure 3). These septums delimited groups of axons which had been remyelinated by oligodendrocytes and Schwann cells. Although the type of cell responsible for remyelination was not quantitated, Schwann cells clearly predominated. In spite of the chronicity of this process, axons were close together, indicating that the glial reaction was mild.

Several important changes at this time were indicative of recurrent demyelinating activity. First, sections from the same spinal cord that contained completely remyelinated plaques also showed foci of inflammation, renewed macrophage activity, and acute demyelination (Figure 4). Second, inflammation and demyelination appeared in areas already remyelinated by Schwann cells. In such areas, lesions of varying severity were observed. Perivascular cuffs of mononuclear cells were surrounded by naked axons that maintained a typical relationship with parent Schwann cells; intermingled were other nerve fibers still surrounded by myelin (Figure 5). In other lesions, entire groups of axons had lost their myelin, and these areas gradually merged with the surrounding parenchyma, which contained myelinated fibers (Figure 6). In the most advanced lesions only naked axons could be found (Figure 7). The majority of such axons were

still in contact with Schwann cells, clearly demonstrating that demyelination had occurred in a remyelinated area. Myelin-debris–laden macrophages were quite numerous in these areas. Thus, it appeared that recurrent demyelination was associated with a more intense macrophage reaction than that during the initial episode of myelin breakdown.

Spinal cord sections at 163 days showed minimal inflammation and demyelinating activity. In extensive areas of the lateral and anterior columns (the entire thickness of the white matter was often involved) there was evidence of earlier demyelination (Figure 8). The inner portion of the columns showed mild remyelinating activity, mainly with CNS myelin. These areas were sharply demarcated from the outer portions, where there was more effective remyelination. Closer observation demonstrated that many Schwann cells participated here in the repair process, although they were less numerous than earlier. In addition, axons were not as close as they were earlier, indicating increased glial reaction.

Electron Microscopy

In the early phase of this infection (Days 16, 24, 31, and 44) there was acute primary demyelination as described in SJL mice.[3] Stripping and vesicular disruption of myelin were often encountered in the midst of large groups of naked axons. These always displayed normal morphologic features (Figure 9). Inflammatory cells were numerous, and most appeared to belong to the lymphocyte series, but a modest plasma cell response was present. Larger cells with irregular contours and cytoplasmic dense bodies, probably representing macrophage precursors, were also seen in moderate numbers. Where demyelination was active, macrophages with abundant lipid droplets and myelin debris were present, but macrophages were never as numerous as they were in SJL mice.[3]

By Day 44, numerous Schwann cells enclosing naked axons were seen deep in the white matter. At this time most axons were simply surrounded by Schwann cell cytoplasm rich in rough endoplasmic reticulum and resting on a basement membrane. However, some axons were already invested by myelin sheaths, having a normal anatomic relationship with parent Schwann cells (Figure 10). Individual axon–Schwann-cell complexes were surrounded by an enlarged extracellular space that was rich in collagen fibers and astroglial processes. Such processes were packed with glial fibrils and were sometimes invested by complete or incomplete basement membranes. Glial processes with normal morphologic features were frequently seen in close vicinity to Schwann cells without interposition of a periglial basement membrane (Figure 10).

Schwann cell remyelination was extensive at Day 84 when large chronic lesions with little or no demyelinating activity were essentially completely remyelinated. In the lateral columns, adjacent to the anterior root entry zone, the majority of axons were remyelinated by Schwann cells with their myelin sheaths having a periodicity of peripheral myelin. The smallest axons remained unmyelinated, but most, nevertheless, were surrounded by Schwann cell cytoplasm. The interaxonal space was rich in collagen fibers (Figure 11). Numerous long, slender, and irregular profiles devoid of basement membranes were present in the extracellular space. These often formed a swirling pattern and were probably of fibroblastic origin. At this time, glial processes were less numerous than earlier and were frequently surrounded by a basement membrane (Figure 11). Some nerve fibers among axons that were surrounded by Schwann cells appeared to be in contact with electron-dense processes that were rich in microtubules but lacking a basement membrane. Some of these processes had laid down a few abnormal myelin lamellas around contacted axons; therefore, they probably were of oligodendroglial origin (Figure 12). Schwann cell remyelination of central axons was also observed in the anterior columns adjacent to and at some distance from the anterior median sulcus. At this site, many of the axons remained naked and were only surrounded by reactive glial processes.

A dramatic change at Day 84 was the appearance of fresh demyelinating activity (Figure 13). As earlier, this activity was strictly related to meningeal and perivascular infiltrates, but now it was seen together with chronic, quiescent lesions that had undergone demyelination earlier and had been remyelinated by Schwann cells.

It was particularly interesting to find acute inflammation and demyelinating activity in remyelinated areas. In such lesions numerous inflammatory cells and macrophages were in close proximity to naked axons (Figure 14). Most of these had a normal axoplasm and were surrounded by Schwann cell cytoplasm that often showed a hypertrophic endoplasmic reticulum and increased electron density. Degenerated myelin often showing vesicular disruption was observed in areas of acute demyelination (Figure 15). Schwann cell processes often had an irregular, undulating contour with redundant, duplicated basement membranes (Figure 16, inset). Astroglial processes with and without basement membranes were often present in these areas (Figures 14 and 16).

At 163 days, approximately equal numbers of axons were remyelinated by Schwann cells and oligodendrocytes in the outer portions of spinal columns. The two different populations of myelinated axons were clearly dis-

tinguishable because of the conspicuous difference in myelin sheath thickness. Axons remyelinated by Schwann cells were, in fact, surrounded by myelin that was 2 to 5 times thicker than myelin generated by oligodendrocytes (Figure 17). Axons myelinated by Schwann cells were generally arranged in lobules interspersed between areas contaning CNS myelin. The interface between peripheral and central type myelin consisted of glial processes which were often covered by a basement membrane. The glial processes were also numerous among axons myelinated by oligodendrocytes, and they contained abundant glycogen granules and densely packed glial filaments (Figure 17).

In the inner portions of spinal cord columns, all axons were ensheathed by CNS myelin. Numerous oligodendrocytes were present in these areas, often next to one another, suggesting that they had proliferated (Figure 18). Contacts between oligodendrocytes and their respective axons could be observed (Figure 18, inset). Little or no demyelinating activity was noted at this time.

Discussion

The present study has revealed that extensive Schwann cell remyelination and recurrent episodes of demyelination occur in the CNS of outbred mice infected with a tissue-culture–adapted TMEV isolate, WW virus. Both findings have relevance to multiple sclerosis, which is characterized in large part by its relapsing course. Schwann cell remyelination in this disease has been the focus of several recent studies.[12–14] Schwann cell remyelination of CNS axons has also been identified in a number of different experimental animal models, including EAE.[15–21] However, Schwann cell remyelination occurred to a moderate extent in those conditions. In WW-virus–infected mice, Schwann cell participation assumed a predominant role in the regeneration of CNS myelin; and by Day 84, axons remyelinated with peripheral myelin constituted the majority of all remyelinated fibers. Peripheral myelin was first detected in the vicinity of the anterior roots, and later it extended for a considerable distance into the white matter. This suggests that most of the Schwann cells probably originated in the root zone. However, it is also possible that some of these cells were derived from intrinsic Schwann cell elements that are known to normally exist in the CNS.[22]

The way in which Schwann cells migrate into the CNS is poorly understood. Blakemore and co-workers have suggested that Schwann cells enter the CNS by transit through a damaged subpial glial limiting membrane, a barrier that normally separates central and peripheral elements.[17–19] In-

jury to this membrane in inflammatory conditions is probably caused by inflammatory cells or their products. Such infiltrates are more extensive in TMEV infection in SJL mice, but Schwann cells were not observed to participate in CNS remyelination in these animals.[3] Leptomeningeal mononuclear cell infiltrates are also extensive in chronic, relapsing EAE, but there is only modest Schwann cell remyelination.[20,21] Therefore, some other factor is probably responsible for the extensive Schwann cell activity in TMEV infection that we have now observed in Swiss mice (infected with tissue-culture–adapted WW virus) and C3H/He mice (infected with brain-derived DA virus).[11] A common denominator in both these TMEV infections is the slow evolution of the demyelinating process, in which only moderate glial and macrophage responses occur. In contrast, the severe acute demyelinating lesions in TMEV infection in SJL mice and in EAE are followed by prominent gliosis. Perhaps the cellular response, particularly gliosis, impedes contact between Schwann cells and axonal membranes, an event necessary for Schwann cell proliferation and myelin production.[23–25] Therefore, prevention of Schwann-cell–axonal contact by gliosis may be more important than the postulated direct contact inhibition between plasmalemmal surfaces of astrocytes and Schwann cells.[17–19]

In addition to gliosis, inflammatory cells, and particularly macrophages, may interfere with remyelination. In contrast to the present results, SJL mice infected with brain-derived DA virus showed only minimal remyelination.[3] These animals had extensive inflammatory cell infiltrates, including many macrophages, and demyelinating lesions lasting for as long as 8 months. The severe demyelinating lesions in SJL mice may be related, in part, to intrinsically enhanced macrophage activity in this strain.[26] This notion is supported by the demonstration that myelin basic protein can be degraded by neutral proteases, including plasminogen activator, secreted by stimulated macrophages.[27] Thus, macrophages which may play a role in initiating myelin injury may also interfere with the formation of new myelin. On the basis of these observations, it is possible that the intensity of the inflammatory and glial responses during demyelination may have a profound influence on the potential for remyelination.

It was of interest that astrocytic processes in WW-virus–infected mice, even when unprotected by a basement membrane, showed no degenerative changes when they were adjacent to Schwann cells. This observation is also at variance with the work of Blakemore et al involving experimental remyelination in rat spinal cord after administration of lysolecithin, 6-aminonicotinamide, or x-irradiation.[17–19] In those studies it was suggested that Schwann cells may have a toxic effect on astrocytes unprotected by a

basement membrane. In agreement with our observations, this type of an effect also was not detected in relapsing EAE.[28]

Another important finding was the discovery of recurrent demyelinating activity later in this infection. Recurrent demyelination was suggested by the simultaneous presence of acute demyelinating lesions occurring together with chronic, quiescent ones in the same section or in different areas of the same spinal cord. The occurrence of acute lesions with chronic lesions has been considered as evidence of recurrent demyelination in chronic, relapsing EAE.[21,29-31] However, the fact that Schwann cells remyelinated extensive portions of the spinal cord in WW-virus–infected mice has provided a basis for more conclusive proof that there were recurrent episodes of myelin breakdown. Initially, most of the areas containing PNS-remyelinated axons were devoid of inflammation. Thus, the subsequent appearance of naked axons ensheathed by Schwann cells in association with inflammatory cell infiltrates and debris-laden macrophages clearly demonstrates that there was at least another bout of myelin degeneration. In this connection the first appearance of spastic paralysis coincided with this recurrent demyelinating activity. The lack of clinical disease during the earlier phase of demyelination highlights the difficulty in correlating clinical signs with pathologic involvement in experimental demyelination. Some of the early investigators of EAE also recognized this discrepancy in animals undergoing acute EAE.[32-34]

The presence of recurrent demyelinating activity enhances the relevance of this experimental infection as an animal model of multiple sclerosis.[35] It is also possible that Schwann cell remyelination of central axons as demonstrated in this study will prove to be an important mechanism of CNS myelin repair in virus-induced demyelinating diseases. It remains to be determined whether such extensive remyelination will have functional significance.

Acknowledgments

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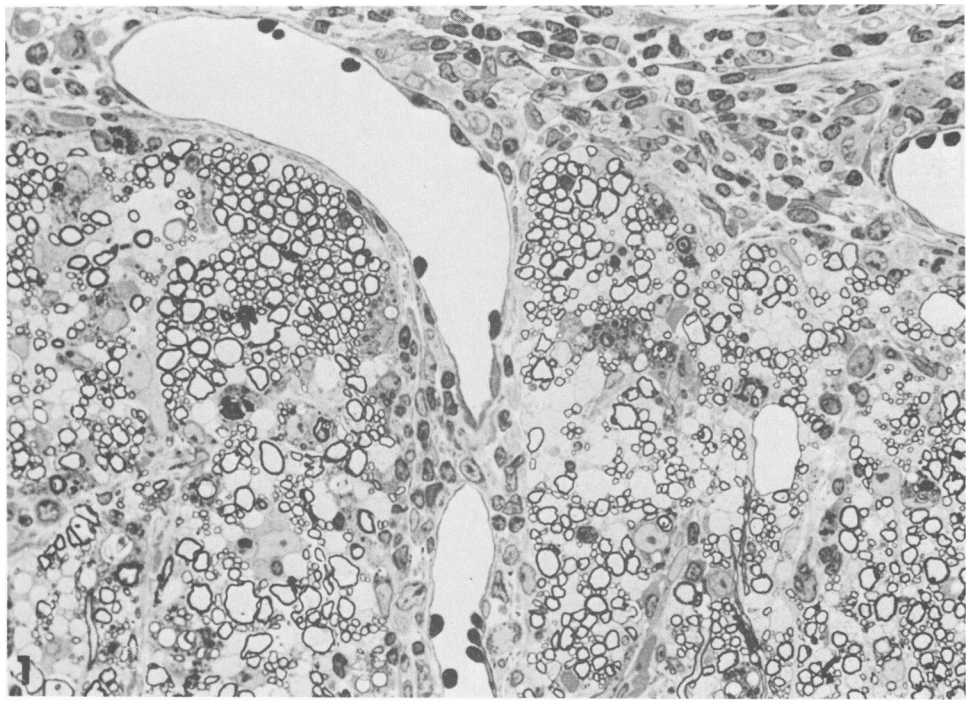
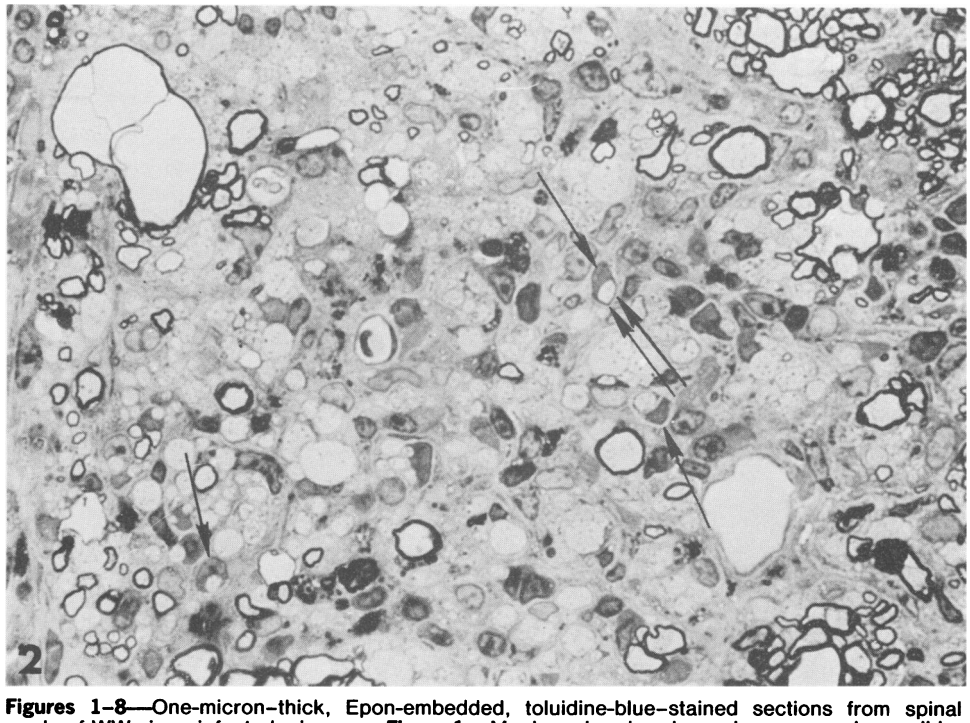
References

1. Theiler M: Spontaneous encephalomyelitis of mice: A new virus disease. Science 80:122, 1934
2. Lipton HL: Theiler's virus infection in mice: An unusual biphasic disease process leading to demyelination. Infect Immun 11:1147–1155, 1975
3. Dal Canto MC, Lipton HL: Primary demyelination in Theiler's virus infection: An ultrastructural study. Lab Invest 33:626–637, 1975
4. Rivers TM, Schwentker FF: Encephalomyelitis accompanied by myelin destruction experimentally produced in monkeys. J Exp Med 61:689–702, 1935
5. Lampert P, Carpenter S: Electron microscopic studies on the vascular permeability

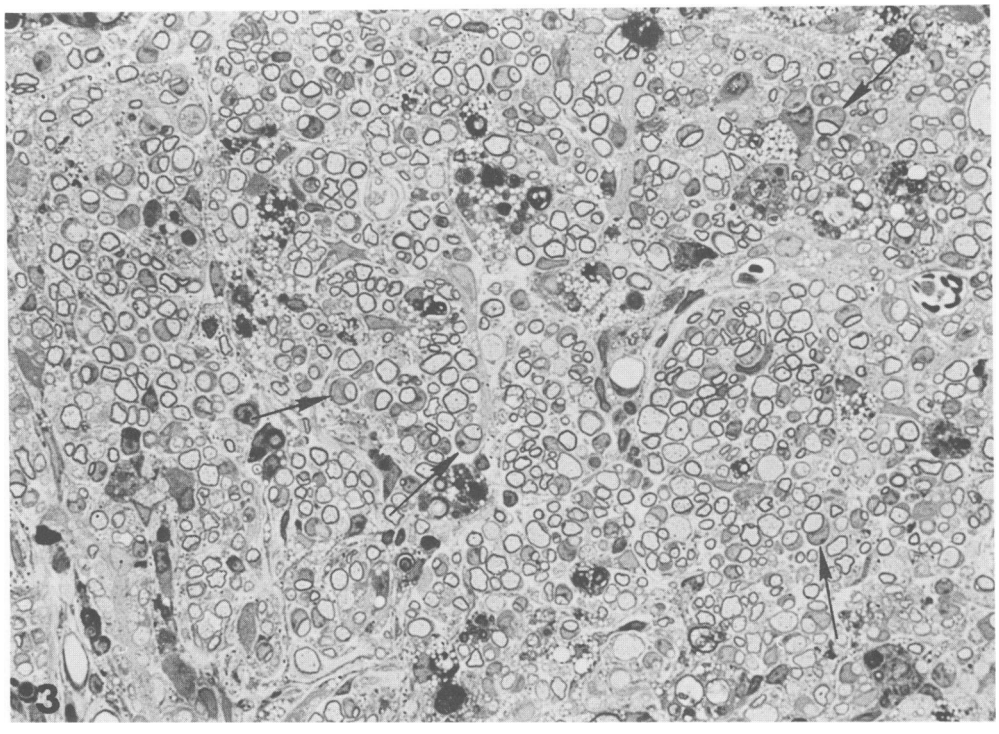
and the mechanism of demyelination in experimental allergic encephalomyelitis. J Neuropathol Exp Neurol 24:11–24, 1965
6. Alvord EC: Acute disseminated encephalomyelitis and allergic encephalopathies, Handbook of Clinical Neurology. Vol 9. Edited by PJ Vinken and GW Bruyn. Amsterdam, North-Holland Publishing Co., 1970, pp 500–571
7. Dal Canto MC, Wiśniewski HM, Johnson AB, Brostoff SW, Raine CS: Vesicular disruption of myelin in autoimmune demyelination. J Neurol Sci 24:313–319, 1975
8. Paterson PY: Neurimmunologic diseases of animals and humans. Rev Infect Dis 1:468–482, 1979
9. Lipton HL, Dal Canto MC: Theiler's virus-induced demyelination: Prevention by immunosuppression. Science 192:62–64, 1976
10. Lipton HL, Dal Canto MC: The TO strains of Theiler's viruses cause "slow virus-like" infection in mice. Ann Neurol 6:25–28, 1979
11. Dal Canto MC, Lipton HL: Recurrent demyelination in chronic central nervous system infection produced by Theiler's murine encephalomyelitis virus. J Neurol Sci (In press)
12. Feigin I, Ogata J: Schwann cells and peripheral myelin within human central nervous tissues: The mesenchymal character of Schwann cells. J Neuropathol Exp Neurol 30:603–612, 1971
13. Ghatak NR, Hirano A, Doron Y, Zimmerman HM: Remyelination in multiple sclerosis with peripheral type myelin. Arch Neurol 29:262–267, 1973
14. Prineas JW, Connell F: Remyelination in multiple sclerosis. Ann Neurol 5:22–31, 1979
15. Lampert P, Cressman M: Axonal regeneration in the dorsal columns of the spinal cord of adult rats. An electron microscopic study. Lab Invest 13:825–839, 1964
16. Hirano A, Zimmerman HM, Levine S: Electron microscopic observations of peripheral myelin in a central nervous system lesion. Acta Neuropathol (Berl) 12:348–365, 1969
17. Blakemore WF: Remyelination by Schwann cells of axons demyelinated by intraspinal injection of 6-aminonicotinamide in the rat. J Neurocytol 4:745–757, 1975
18. Blakemore WF, Patterson RC: Observations on the interactions of Schwann cells and astrocytes following x-irradiation of neonatal rat spinal cord. J Neurocytol 4:573–585, 1975
19. Blakemore WF: Invasion of Schwann cells into the spinal cord of the rat following local injection of lysolecithin. J Neuropath Appl Neurobiol 2:21–39, 1976
20. Snyder DH, Valsamis MP, Stone SH, Raine CS: Progressive demyelination and reparative phenomena in chronic experimental allergic encephalomyelitis. J Neuropathol Exp Neurol 34:209–221, 1975
21. Raine, CS, Traugott U, Stone SH: Chronic relapsing experimental allergic encephalomyelitis: CNS plaque development in unsuppressed and suppressed animals. Acta Neuropathol (Berl) 43:43–53, 1978
22. Raine CS: On the occurrence of Schwann cells within the normal central nervous system. J Neurocytol 5:371–380, 1976
23. Wood PM, Bunge RP: Evidence that sensory axons are mitogenic for Schwann cells. Nature 256:662–664, 1975
24. Aguayo AJ, Peyronnard JM, Terry LC, Romine JS, Bray GM: Neonatal neuronal loss in rat superior cervical ganglia: Retrograde effects on developing preganglionic axons and Schwann cells. J Neurocytol 5:137–155, 1976
25. Spencer PS, Weinberg HJ: Axonal specification of Schwann cell expression and myelination, Physiology and Pathobiology of Axons. Edited by S Waxman. New York, Raven Press, 1978, pp 231–239
26. Gallily R, Haran-Ghera N: Immune macrophage functions in high and low cancer incidence strains of mice. Fed Proc 37:1841, 1978

27. Cammer W, Bloom BR, Norton WT, Gordon S: Degradation of basic protein in myelin by neutral proteases secreted by stimulated macrophages: A possible mechanism of inflammatory demyelination. Proc Natl Acad Sci USA 75:1554–1558, 1978
28. Raine CS, Traugott U, Stone SH: Glial bridges and Schwann cell migration during chronic demyelination in the C.N.S. J Neurocytol 7:541–553, 1978
29. Stone SH, Lerner EM II: Chronic disseminated allergic encephalomyelitis in guinea pigs. Ann NY Acad Sci 122:227–241, 1965
30. McFarlin DE, Blank SE, Kibler RF: Recurrent experimental allergic encephalomyelitis in the Lewis rat. J Immunol 113:712–715, 1974
31. Wiśniewski HM, Keith AB: Chronic relapsing experimental allergic encephalomyelitis: An experimental model of multiple sclerosis. Ann Neurol 1:144–148, 1976
32. Alvord EC Jr, Magee KR, Kies MW, Goldstein NP: Clinico-pathologic correlations in experimental "allergic" encephalomyelitis: I. Observations on the early lesion. J Neuropathol Exp Neurol 18:442–446, 1959
33. Bornstein MB: Anti-glial and anti-neuronal factors in experimental allergic encephalomyelitis and multiple sclerosis, Multiple Sclerosis. Edited by F Wolfgram, GW Ellison, JG Stevens, JM Andrews. New York, Academic Press, 1972, pp 119–126
34. Levine S, Sowinski R, Shaw C-M, Alvord EC Jr: Do neurological signs occur in experimental allergic encephalomyelitis in the absence of inflammatory lesions of the central nervous system? J Neuropathol Exp Neurol 34:501–506, 1975
35. Dal Canto MC, Lipton HL: Multiple sclerosis: Animal model: Theiler's virus infection in mice. Am J Pathol 88:497–500, 1977





Figures 1–8—One-micron-thick, Epon-embedded, toluidine-blue-stained sections from spinal cords of WW-virus-infected mice. **Figure 1**—Meningeal and perivascular mononuclear cell infiltrates which are surrounded by numerous demyelinated axons; 24 days after infection. (×390) **Figure 2**—A well-demarcated plaque of demyelination in a quiescent state that shows numerous Schwann cells extending deep into the lesion (*arrows*). Note the typical Schwann-cell–axonal relations. *Double arrow* indicates a thin myelin sheath; 44 days after infection. (×580)



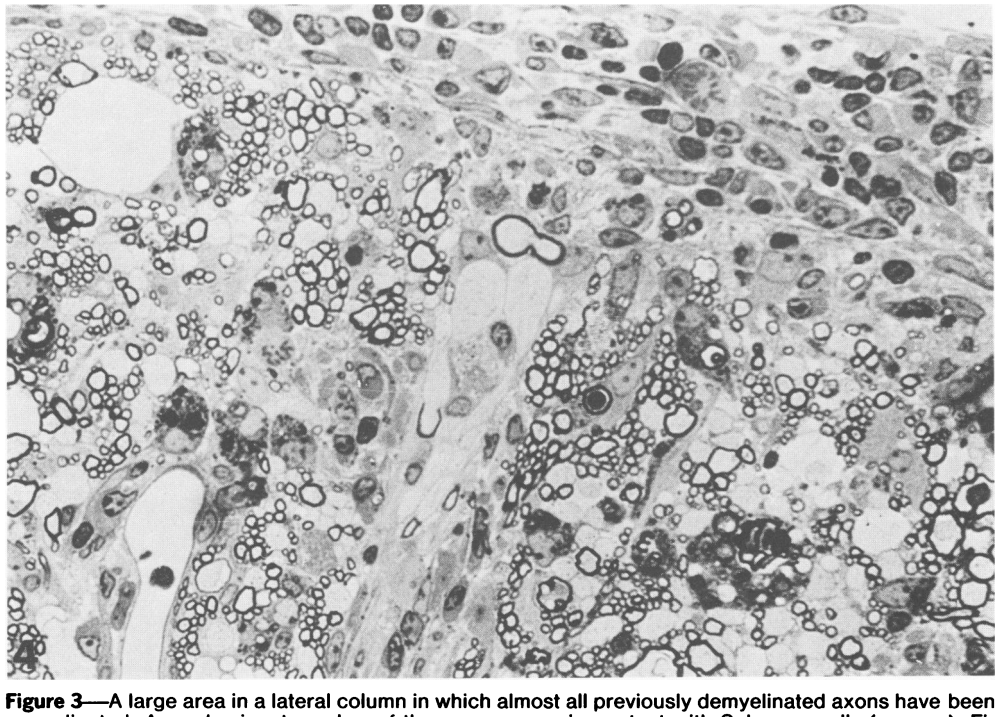
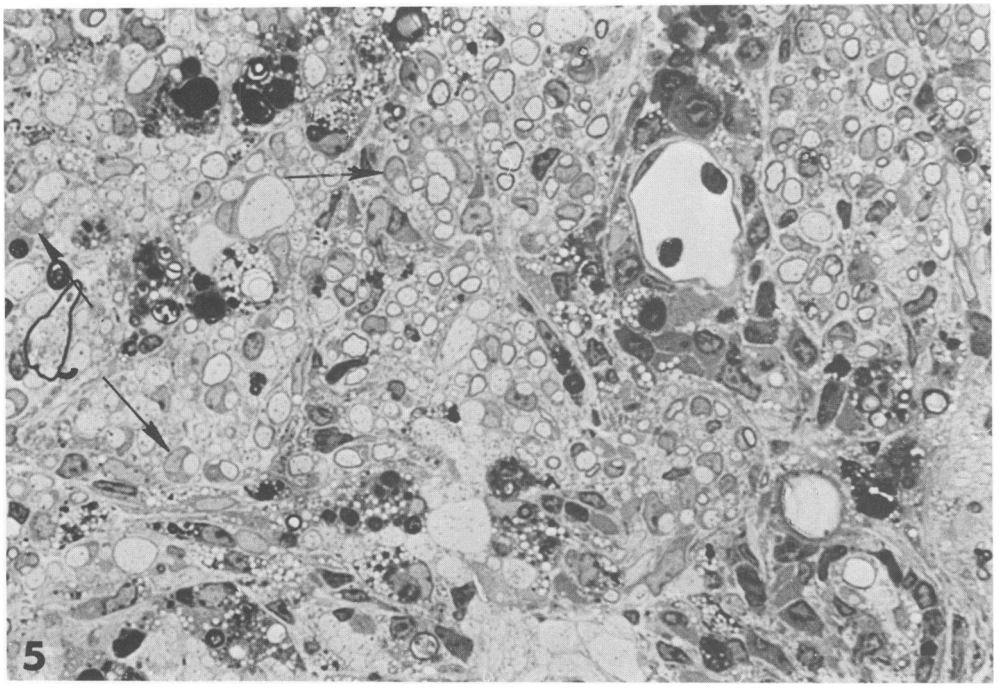


Figure 3—A large area in a lateral column in which almost all previously demyelinated axons have been remyelinated. A predominant number of these axons are in contact with Schwann cells (*arrows*). Fibrous septums are delimiting lobular groups of axons, and each group may contain both central and peripheral type myelin. Gliosis is mild; 84 days after infection. (×400) **Figure 4**—A section from the same spinal cord as in Figure 3 that shows an acute demyelinating lesion with inflammatory cells and some myelin debris-laden macrophages. (×490)



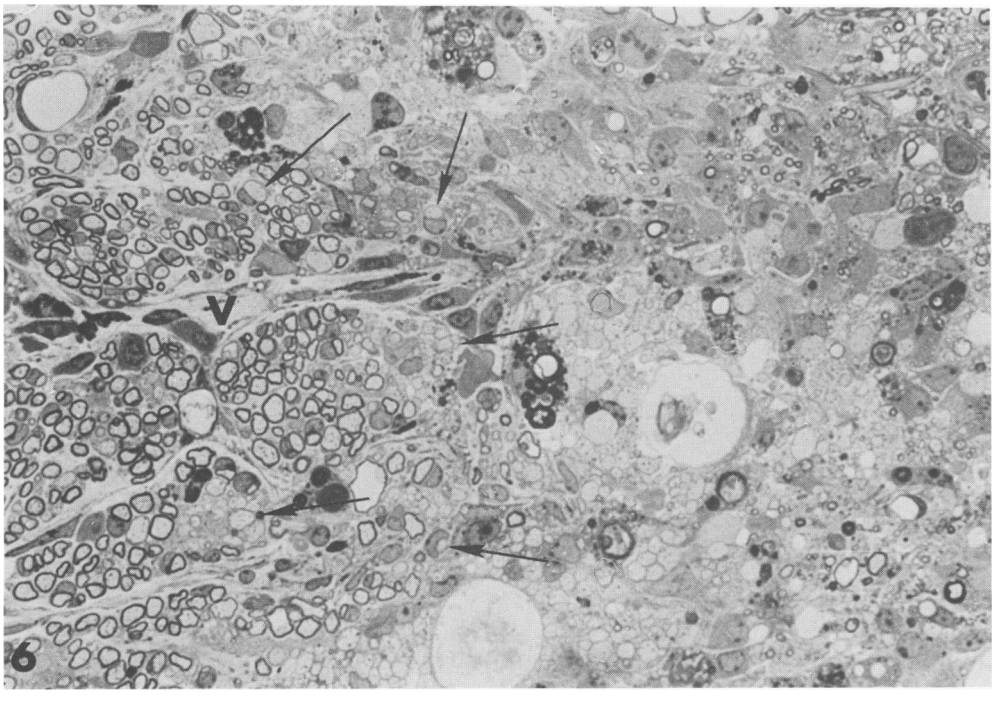
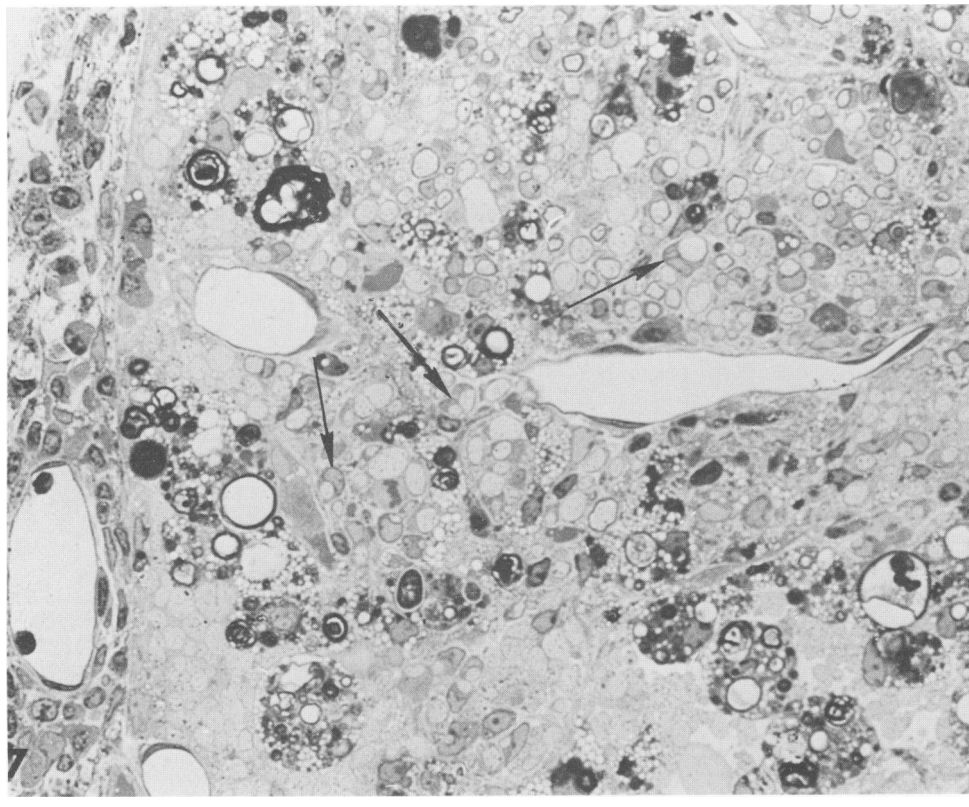


Figure 5—Perivascular inflammatory infiltrates surrounded by demyelinated axons (*right lower corner*). Many axons in the upper two thirds of the field are ensheathed by Schwann cells (*arrows*) and are intermingled with axons still having their original myelin; 84 days after infection. (×430) **Figure 6**—An entire lobule of a remyelinated plaque that has been demyelinated, containing many naked axons clearly attached to Schwann cells (*arrows*). The demyelinated area is close to a tangentially cut vessel (*V*) and inflammatory cells. The left portion of the field shows the quiescent portion of this lesion with its remyelinated axons; 84 days after infection. (×500)



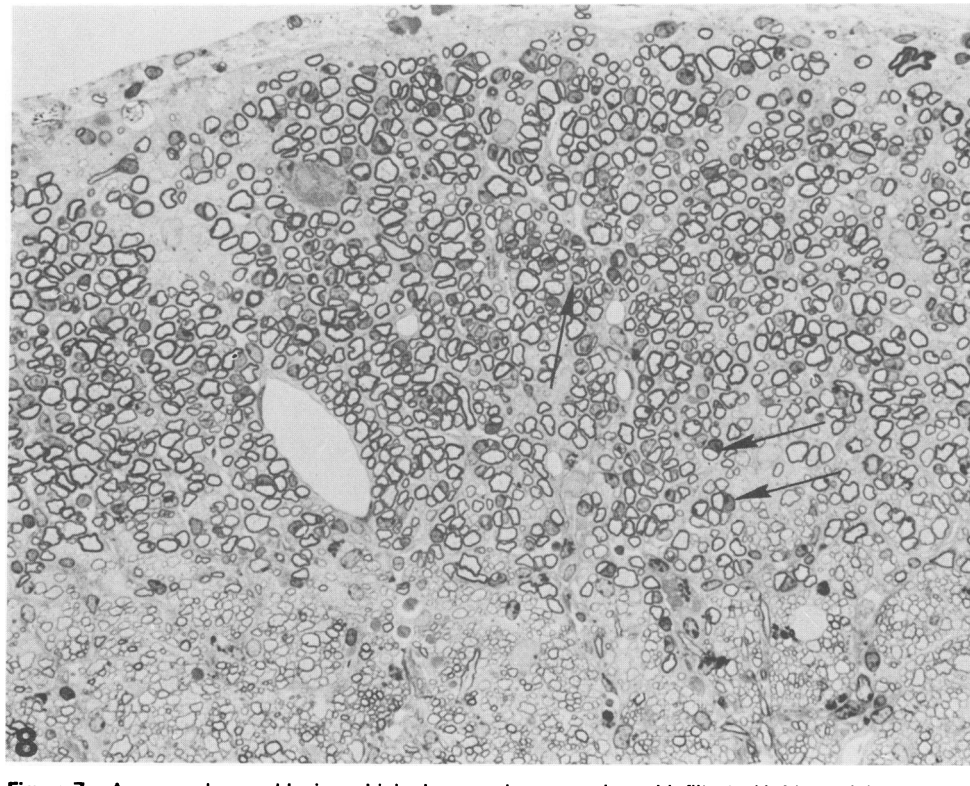
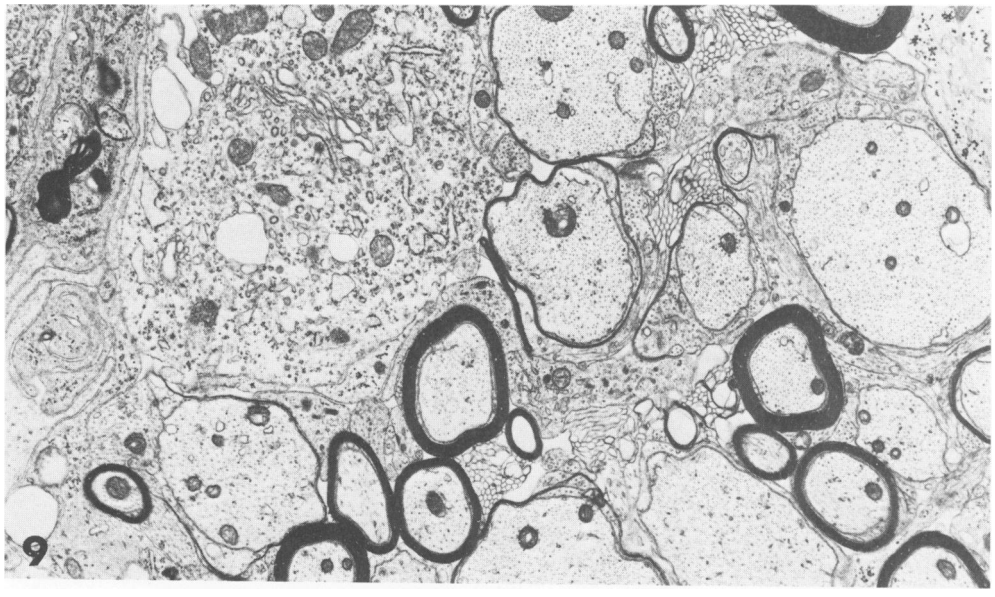


Figure 7—A more advanced lesion which shows a dense meningeal infiltrate (*left*) overlying a totally demyelinated lesion in which most axons are attached to Schwann cells (*arrows*). Numerous myelin debris-laden macrophages are infiltrating the area, indicating that this is an active process; 84 days after injection. (×590) **Figure 8**—Almost the entire thickness of a lateral column is seen. The upper half shows a portion of an extensive area of remyelination in which several Schwann cells are recognizable (*arrows*). The lower half shows numerous naked axons, some of which have been remyelinated by central myelin. Note the clear demarcation between the two areas and the lack of activity at this time; 163 days after infection. (×410).



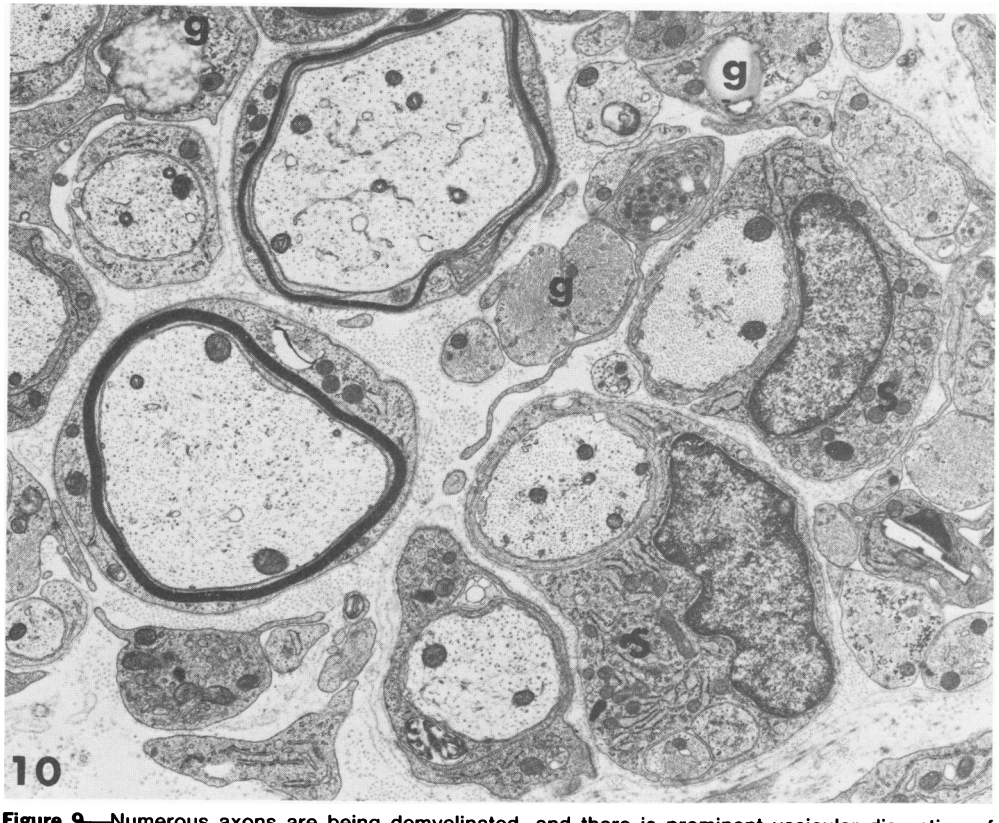


Figure 9—Numerous axons are being demyelinated, and there is prominent vesicular disruption of myelin; 24 days after infection. (×77,000) **Figure 10**—All of the axons in this field are ensheathed by Schwann cells (*S*) whose processes are spiraling around the axons. Two of the larger axons have already been ensheathed with thin myelin. In addition, there are numerous glial processes (*g*), with and without basement membranes, and they are rich in glial filaments and show no degenerative change. The extracellular space contains collagen fibers; 44 days after infection. (×12,700)

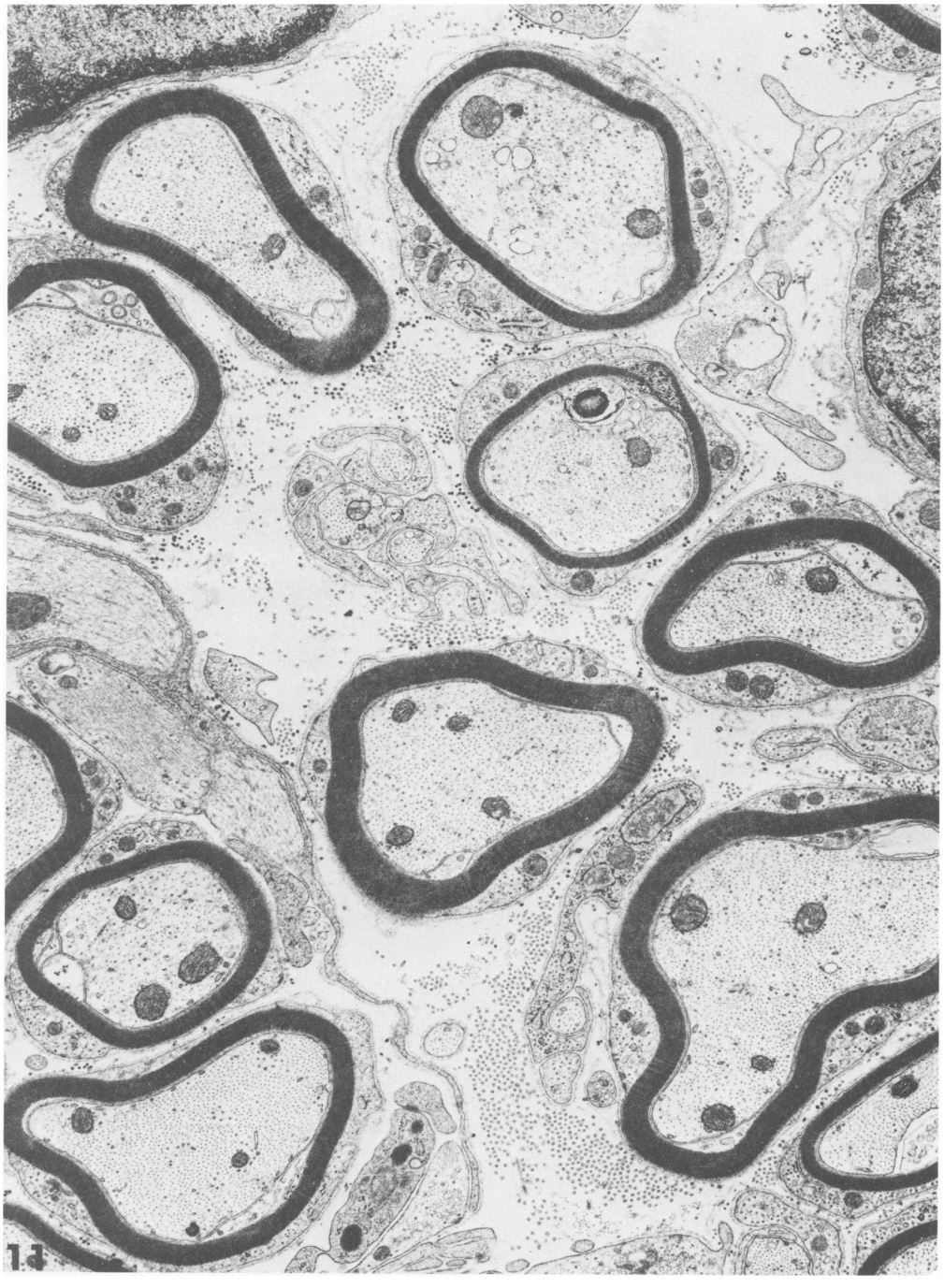
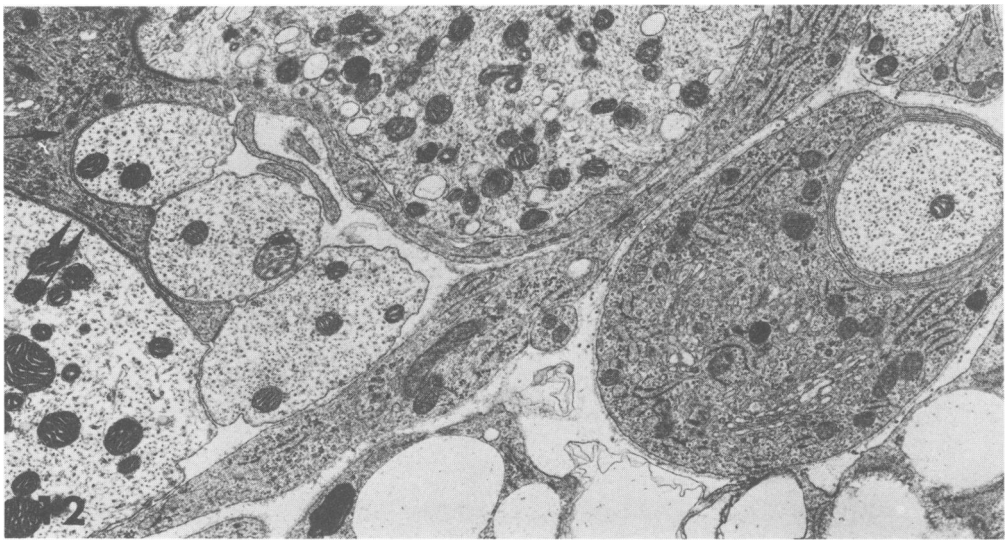


Figure 11—All of the axons in this area have been remyelinated by Schwann cells. Note the normal appearance of the Schwann cell cytoplasm resting on a basement membrane. Some of the glial processes are surrounded by a basement membrane, while others are not, but nonetheless show normal features; 84 days after infection. (×13,000)



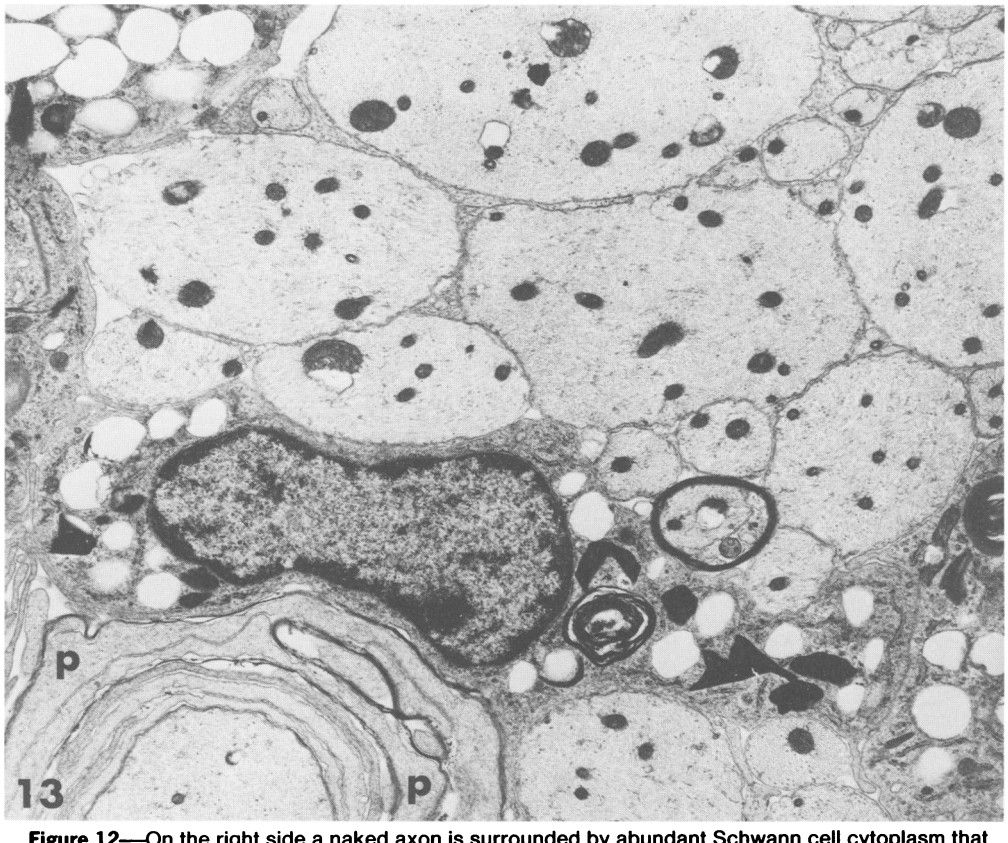
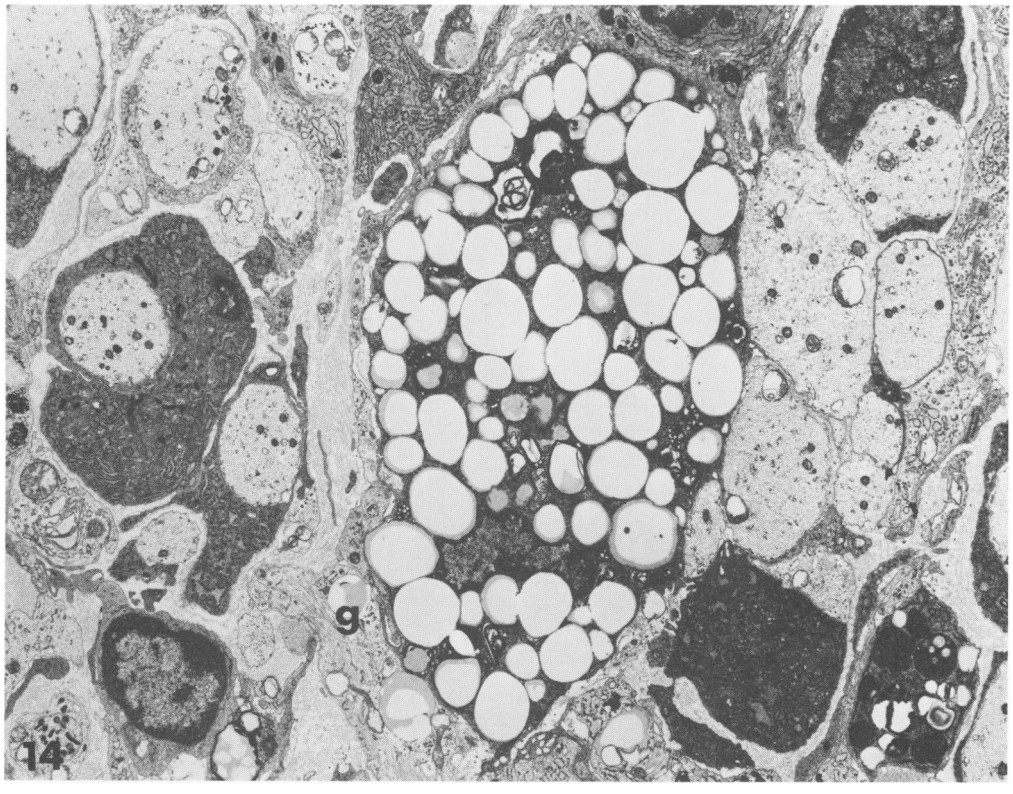


Figure 12—On the right side a naked axon is surrounded by abundant Schwann cell cytoplasm that is resting on a basement membrane; note the spiraling of the Schwann cell around this axon. On the left side a portion of electron-dense cytoplasm rich in microtubules (*arrow*) has contacted several naked axons and shows membrane thickening suggestive of myelin formation (*double arrows*). The overall appearance of this cell is that of an oligodendrocyte. (×15,000) **Figure 13**—An area with recent demyelinating activity shows stripping of myelin by mononuclear cell processes (*p*), numerous naked axons, and macrophages containing myelin debris. Areas such as this were seen at 84 days after infection in spinal cords also showing completely remyelinated plaques. (×10,600)

Figure 14—A large macrophage laden with myelin debris and lipid droplets is surrounded by a number of naked axons, many of which are still in contact with Schwann cells. Many of the Schwann cells show irregular contours and variably dense cytoplasm. Note the presence of glial processes rich in glial fibrils (*g*) and the extracellular space filled with collagen bundles. Portions of plasma cells are seen at the top of the field. (×7200)

Figure 15—The center of the field is occupied by disorganized arrays of degenerating myelin lamellas, including some vesicular type degenerative products. Naked axons in contact with Schwann cells are present at the top and bottom; 84 days after infection. (×12,900)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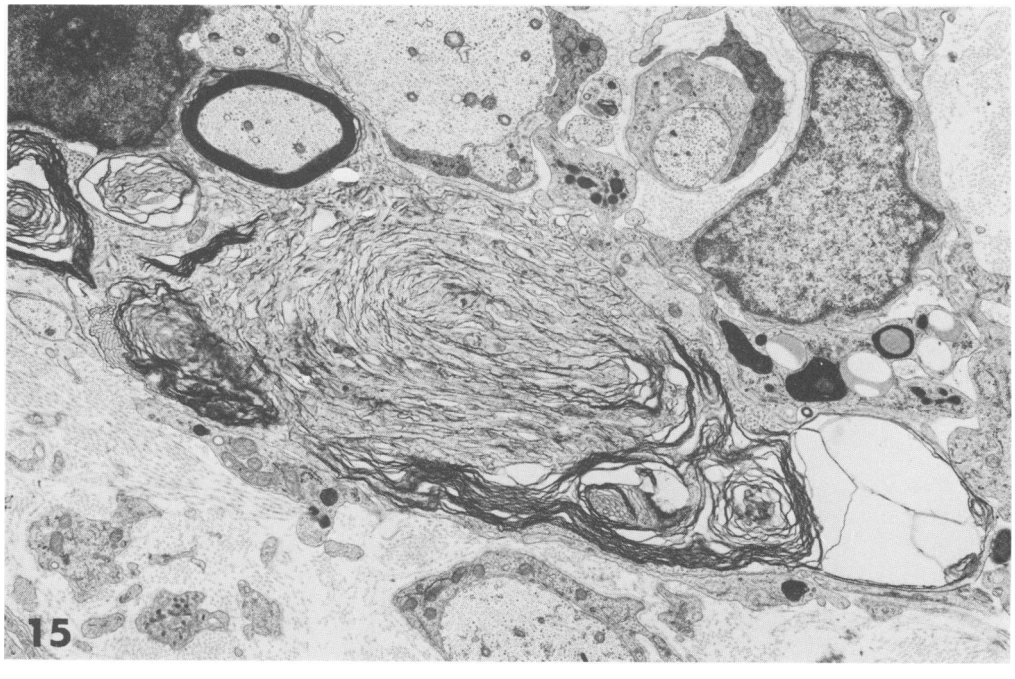
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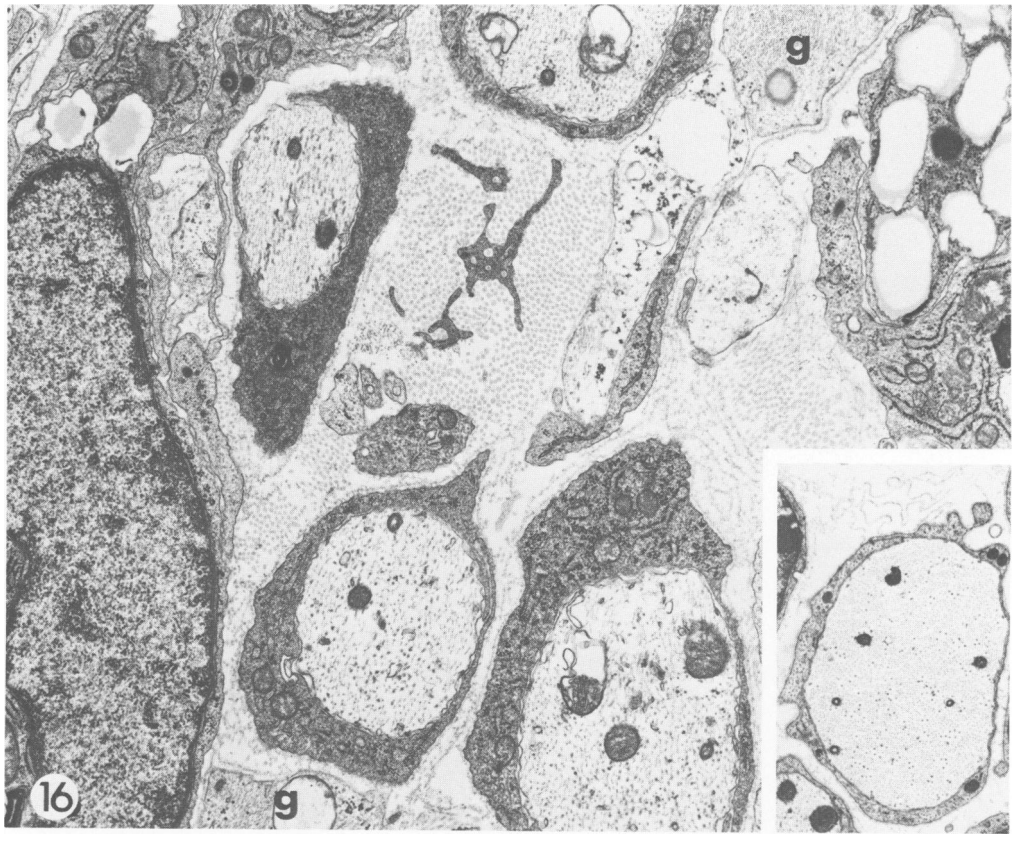
Figure 16—This area shows four large demyelinated axons still surrounded by Schwann cells and an extracellular space rich in collagen fibers. Glial processes (*g*) are present, and the one at the top has no basement membrane on the side of the axon but a basement membrane on the side of the macrophage. (×7900) **Inset**—A naked axon surrounded by Schwann cell cytoplasm. Note the undulating cell contours and abundant duplicated basement membrane; 84 days after infection. (×14,800)

Figure 17—A mixed population of axons is shown. Four axons (*a*) are surrounded by thick myelin sheaths and Schwann cell cytoplasm resting on a basement membrane. Other axons are surrounded by thinner myelin of oligodendrocyte origin. Astrocytic processes are present among the axons; 163 days after infection. (×12,700)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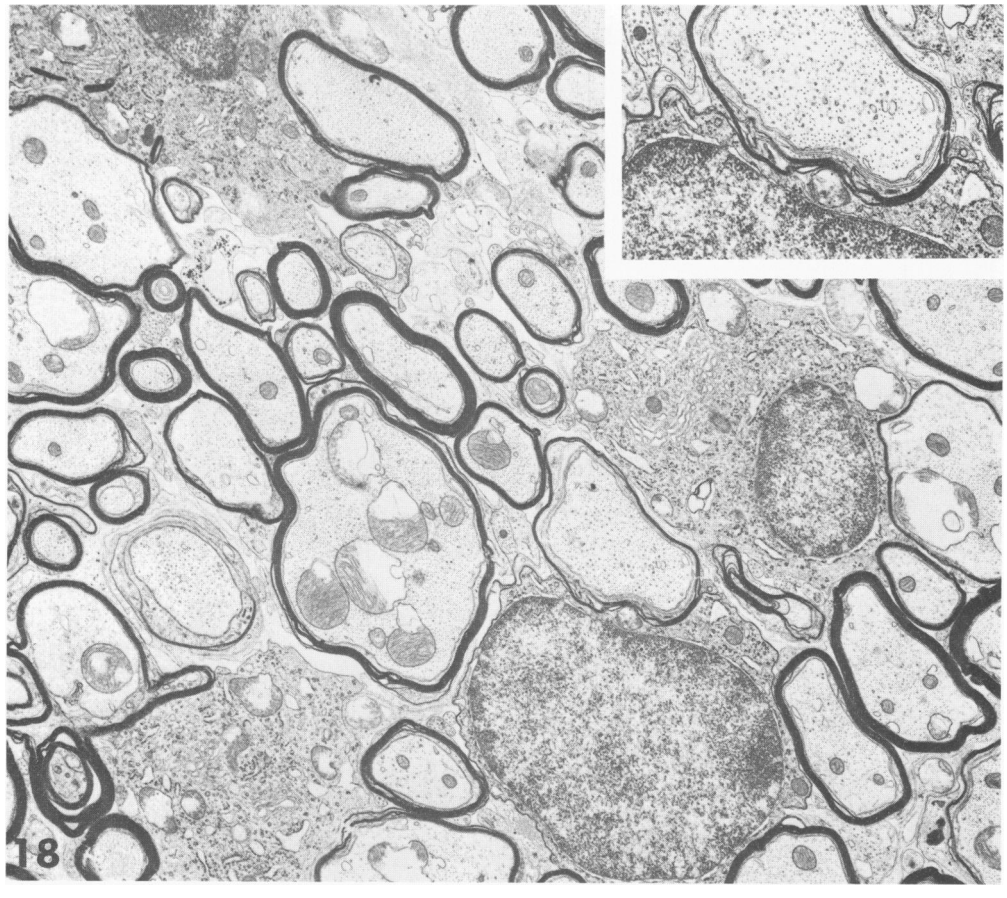

Figure 18—Four oligodendrocytes are seen in the inner portion of a lateral column. All the axons show remyelination by central myelin. (×7200) **Inset**—A magnified view of a contact between a myelinating oligodendroglial cell and its axon; 163 days after infection. (×15,000)